MH 32 D7

February 16th, 1948

Prof. Dr. Felix Haurowitz,
Istanbul Universitesi
Istanbul, Turkey

Dear Haurowitz,

Thank you for sending me your paper with the referee's comments and your views.

If I interpret the referee's comments correctly on the basis of my own experience, he seems to be talking about antigens which behave, in the precipitin reaction, like single substances. In these cases it has been useful to assume that all of the added antigen is precipitated in the region of antibody excess and in every instance in which it has been possible to check this point it has been found to be true (thyroglobulin, hemocyanin, dye egg albumin, blood group A substance). You forget that tests of the supernatants for antigen and antibody, if properly carried out with avoidance of inhibitory concentrations, give confirmatory evidence on this point. If an antigen is a mixture and there are antibodies to more than one antigen present, the equivalence zone usually gives tests for antigen and antibody. I do not see that globulin anti-globulin systems present any special difficulty provided the antigen is electrophoretically and ultracentrifugally homogeneous and the supernatant tests show a proper equivalence zone (see Treffers' and my recent papers on antiproteins in horse sera, J. Exp. Med., 1947. 86. 77, 83. 95. There you will also find a nucleoprotein that behaves as if it were all precipitated).

With regard to azoproteins, I do not feel that they help matters very much, now that we know as much as we apparently do. The trouble is that they, too, are mixtures. Is all the color in your azo-globulin precipitated by excess antibody? If not, is the color:nitrogen ratio of the precipitated portion the same as that of the unprecipitated part, or as that of the unfractionated whole? I do not recall any of your studies in which this point was considered, and it could surely introduce errors as large as caused by the unjustified assumption that all of a homogeneous antigen was precipitated. Such errors would be particularly large in the region of antigen excess and it would not be safe to assume that a direct analysis of the supernatant for antigen could be avoided.

I do not recall any instance in which I have proved a natural, homogeneous antigen to be only partly precipitated -- the instances you name are azo-compounds which are mixtures even when a homogeneous starting material is used. Streptococcus nucleoprotein is also only partly precipitated, but it is probably grossly inhomogeneous.

I would interpret the last sentence of the referee's comment to mean that antigenic globulin can be differentiated from antibody globulin and determined in its presence. This was done in J. Exp. Med., 1941, 73, 125, 293; 1942, 75, 135. and in the reverse system quoted above. If you do not agree, that is your privilege, but you should not make a categorical statement contradicting published evidence without referring to the original material and giving your own evidence. Perhaps you merely tried to present this work in a form that was too brief.

It seems to me that you would need to rewrite the paper to make it really publishable, even if it involves an extra page or two to present your own views properly and to explain their differences from those already in print. Another and shorter way would be merely to present the work as a method of estimating antiprotein without theoretical discussion and reference only to similar publications of others.

I do not see any reason why you should need to send your papers to me before publication.

With all good wishes,

Sincerely yours,

MH:jm

Michael Heidelberger